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(71) Applicant: PITMAN-MOORE, INC. [US/US]; P.O. Box 207, 1401 South Third Street, Terre Haute, IN 47808 (US).

(72) Inventors: SEELY, James, E. ; 203 Hoover, Louisville, CO 80017 (US). HULBERT, Matthew, H. ; 45 Heritage Drive, Terre Haute, IN 47803 (US). RICHEY, Carl, W., Jr. ; 26 17th Avenue, Longmont, CO 80501 (US). AUER, Henry, E. ; Apartment 1, 137 Templeton Parkway, Watertown, MA 02172 (US).

(74) Agent: ERNST, Barbara, G.; Rothwell, Figg, Ernst & Kurz, 555 13th Street, N.W. #701 East, Washington, DC 20004 (US).

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(54) Title: LYOPHILIZED SOMATOTROPIN FORMULATIONS

(57) Abstract

Somatotropin solutions which remain clear for extended periods and which remain clear when subjected to mechanical agitation are prepared by mixing a lyophilized somatotropin composition containing about 1 part somatotropin per 2 to 8 parts arginine HCl on a weight basis, wherein the pH of the composition prior to lyophilization was about 7.2 to about 8.5, and a biocompatible diluent which comprises EDTA and a nonionic surfactant. If the pH of the somatotropin composition prior to lyophilization was between 7.2 and 7.8, the diluent further comprises a buffer; otherwise the diluent optionally further can comprise a buffer or nonbuffering agent.

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lyophilized somatotropin formulations

BACKGROUND OF THE INVENTION

The present invention relates to novel solutions of somatotropins which remain clear for an extended period of time and which remain clear when subjected to mechanical agitation, and to kits for making the solutions.

Somatotropins, also known as growth hormones, are polypeptide hormones secreted by the pituitary glands of many animal species. These hormones are valuable for a number of therapeutic uses, and compositions comprising somatotropin can be administered in the treatment of pituitary deficiency in humans and gastrointestinal bleeding or to promote the healing of bone fractures and accelerate the healing of contusions and other wounds. Somatotropins also are useful in promoting meat and milk production in animals when administered through various drug-releasing devices or by injection. (See E.J. Turman, "Some Effects of Pituitary Anterior Growth Factor" Thesis: Purdue University, April, 1953; L.J. Machlin, J. Anim. Sci. 35: 794-800 (1972); T.R. Kasser et al., J. Anim. Sci. 53: 420-426; L.J. Machlin, J. Dairy Sci. 56: 575-580 (1973)).

It frequently is desirable to work with and administer proteins such as somatotropins in the form of a solution. However, dissolved proteins, such as

somatotropin, can be adsorbed at hydrophobic interfaces, thus causing secondary reactions. For example, "denaturing", i.e. a change in the shape of the adsorbed somatotropin molecules can occur. In addition, aggregation of adsorbed somatotropin molecules can take place to give soluble or insoluble polymeric forms. This aggregation will manifest itself, for example, as turbidity of the solution or as biological inactivation of the somatotropin protein on stirring or shaking of the aqueous solutions (See A.F. Henson et al., J. Colloid Interface Sci. 32: 162 (1970)).

When the somatotropin precipitates from solution, the precipitated protein becomes unavailable for administration. Thus, a somatotropin solution is needed which will not become turbid upon stirring or shaking and which will remain clear over extended periods of storage.

Summary of the Invention

In accordance with the present invention, there are disclosed somatotropin solutions which remain clear for extended periods and which do not become cloudy or precipitate when subjected to mechanical agitation. The somatotropin solutions contain a lyophilized somatotropin composition containing about 1 part somatotropin per 2 to 8 parts arginine HCl on a weight basis, the pH of the composition prior to lyophilization within the range of about 7.2 to about 8.5, which is dissolved in a diluent. If the pH of the somatotropin composition prior to lyophilization was less than 7.8, the diluent comprises EDTA, a nonionic surfactant and a buffer. If the pH of the somatotropin

composition prior to lyophilization was 7.8 or greater, the diluent compris s EDTA and a nonionic surfactant and optionally, further can comprise a suitable buffer or nonbuffering agent if desired. If a non-buffering agent is added, desirably a buffer is not also added to the diluent, although both agents can be used.

This invention further provides kits for making the solutions. The kits include a vial containing the lyophilized somatotropin composition containing about 1 part somatotropin per 2 to 8 parts arginine HCl on a weight basis. The kits also include a vial containing a biocompatible diluent comprising EDTA and a nonionic surfactant. In addition, as described above, depending upon the pH of the somatotropin composition prior to lyophilization, the diluent also can comprise a buffer or non-buffering agent. If the pH of the somatotropin composition prior to lyophilization was less than 7.8, the vial containing the diluent further comprises a buffer. At a pH of 7.8 or greater the vial optionally can contain a buffer or nonbuffering agent. The diluent can be added to the lyophilized somatotropin composition and the resultant mixture then shaken to dissolve the somatotropin.

Detailed Description of the Invention

This invention is directed to somatotropin solutions which remain clear for extended periods and which do not become cloudy or precipitate when subjected to mechanical agitation such as shaking or vortex-mixing. A somatotropin solution which remains "clear" is a solution in which the somatotropin does not precipitate after it is dissolved in the diluent. These somatotropin solutions will remain cl ar after

several days of storage, typically at l ast about 5 days. The somatotropin solutions maintain clarity for at least about 5 days when stored, typically, for example, at about 25°C. At slightly higher storage temperatures, the somatotropin solutions maintain clarity, but possibly for a shorter period of time. A solution which remains clear will have an apparent absorbance measurement at 360 nm (O.D. 360) of less than 0.10.

The somatotropin solutions contain a lyophilized somatotropin composition, the pH of which prior to lyophilization was at least about 7.2, generally within the range of from about 7.2 to about 8.5. If the pH of the somatotropin composition prior to lyophilization was less than 7.8, the diluent comprises EDTA, a nonionic surfactant and a suitable buffer to stabiliz the final somatotropin solution. If the pH of the somatotropin composition prior to lyophilization was 7.8 or higher, the diluent comprises EDTA and an nonionic surfactant and, optionally, a buffer or nonbuffering agent. If a non-buffering agent is added to the diluent, desirably a buffer is not also added to the diluent, although both agents can be used. Neither the buffer nor nonbuffering agent need be used if the pH of the somatotropin composition prior to lyophilization was at least 7.8, but either can be provided to adjust or maintain the isotonicity of the resultant somatotropin solution such that it is less hypertonic. This is desirable if the somatotropin solution will be injected into animals. The choice of a suitable additive can be made based on a number of factors, including, for example, cost, presence on th FDA GRAS list, autoclavability, chemical stability,

lack of interaction with the somatotropin and biocompatibility.

The lyophilized somatotropin composition is prepared by dissolving arginine HCl with somatotropin, adjusting the pH of the solution to at least 7.2, preferably from 7.2 to 8.5, and removing any undissolved material by filtration or centrifugation. The somatotropin and arginine HCl solution then is lyophilized using standard procedures known in the art.

The solution to be lyophilized generally contains about 1 part somatotropin per 2 to 8 parts arginine HCl on a weight basis and preferably comprises about 1 part somatotropin per 3 parts arginine HCl. Thus, the solution preferably contains 10 to 150 mg/ml somatotropin and 30 to 450 mg/ml arginine HCl and has a final pH of at least 7.2. Most preferably, the solution to be lyophilized contains 30 mg/ml somatotropin and 90 mg/ml arginine HCl and has a final pH of from 7.8 to 8.5.

The somatotropin which may be employed in the lyophilized somatotropin composition of this invention can be any somatotropin, including natural or recombinant bovine, porcine, human, avian, ovine, or equine somatotropin. Preferably, the somatotropin employed is porcine somatotropin. As used herein, th term somatotropin is intended to include the full length natural or recombinant somatotropin as well as derivatives thereof that have growth-promoting capabilities. Derivatives include biologically active fragments or analogs of the polypeptide hormone, such as delta 7 porcine somatotropin, which has an amino acid sequence corresponding to that of porcine somatotropin, less the first seven amino acids of the matur , full length hormone (described in European

Patent Application Publication No. 0 104 920 to Biogen N.V.). The term "biologically active" as used herein means a polypeptide that, following its administration to a living being, has a demonstrable effect on a biological process of that living being.

The somatotropins employed in the compositions of this invention can be metal-associated somatotropins. Metal-associated somatotropin is produced by the addition of salts of transition metals to an aqueous solution containing the somatotropin. The salts of the transition metals form insoluble complexes with the somatotropin, thus precipitating the somatotropin out of the solution. The metal-associated somatotropins comprise the somatotropin molecules and metal ions such as Zn^{+2}, Cu^{+2}, Co^{+2}, Mn^{+2}, Fe^{+2} or Fe^{+3}. These metal-associated somatotropins contain ligand bonds between the metal ion and the nitrogen atoms of some of the amino acid residues in the somatotropin molecule. The metal-associated somatotropins are used as starting materials for making the lyophilized somatotropin compositions. The metals likely are not associated with the somatotropin once the metal-associated somatotropin is lyophilized, although they are present in the lyophilized somatotropin composition. The presence of the transition metal in the product has been shown to have no significant adverse effect on th bioactivity of the somatotropin when the product is administered to a living being.

The somatotropin solutions of this invention are made by dissolving the lyophilized somatotropin composition with a diluent such that the pH of the final solution is at least about 7.2, typically from about 7.2 to about 8.5, and preferably from about 7.2 to about 8.2. If th pH of th solution is greater

than about 8.5, degradation of the protein can occur. Typically, about 0.5 to about 40 mg lyophiliz d somatotropin composition are provided per ml of diluent. Preferably, the final concentration of the somatotropin solution is about 10 to 30 mg lyophilized somatotropin composition per ml of diluent and most preferably about 20 mg lyophilized somatotropin composition per ml of diluent. The weight of the lyophilized somatotropin composition will be the total of the weight of the arginine HCl and the weight of the somatotropin contained in the lyophilized somatotropin composition.

If the pH of the somatotropin composition to be used in making the somatotropin solution was less than 7.8 prior to lyophilization, the diluent comprises EDTA, a nonionic surfactant and a buffer. Alternatively, if the somatotropin composition had a pH of at least 7.8 prior to lyophilization, the diluent comprises EDTA and a nonionic surfactant and optionally also comprises a buffer or nonbuffering agent. If the diluent comprises a buffer, the pH of the final somatotropin solution will be the same as the pH of the diluent. However, if the diluent is not buffered, the pH of the lyophilized somatotropin composition dissolved in the diluent will determine the pH of the final somatotropin solution.

Desirably, when the pH of the final somatotropin solution is between about 7.2 and about 7.6, the concentration of EDTA provided in the diluent is at least 1.5 mM, and is preferably from about 1.5 mM to about 10 mM. When the final somatotropin solution pH is greater than 7.6, the concentration of EDTA used in the diluent desirably is at least 1.0 mM, and is preferably from about 1.0 mM to about 10 mM.

Surfactants which are suitable for use in the diluent include polyoxyethylene-23 lauryl ther (Brij 35), Tween 80, polyoxyethylene-20 cetyl ether (Brij 58), and other polyoxyethylene nonionic surfactants having similar hydrophilic/hydrophobic balance (HLB). Such nonionic surfactants have been noted in the prior art as stabilizing and preserving activity in purified enzymes. (See T. Kitani et al., Eur. J. Biochem. 119: 177-181 (1981); M. Pritchard et al., Biochem. Biophys. Res. Commun. 100: 1597-1603 (1981); Seely et al., Biochemistry, Vol. 21, No. 14, 3394-3399 (1982)). Generally, the surfactant is present in a concentration ranging from about 0.08% to 2.0%. If the surfactant utilized is Brij 35, the concentration of the Brij 35 is at least 0.08%, and preferably is from about 0.1% to about 0.2%. If the surfactant is Tween 80 or Brij 58, the concentration preferably is from about 0.1% to about 1.0%.

If the pH of the somatotropin prior to lyophilization was at least 7.2 but less than 7.8, a buffer is provided in the diluent to increase the stability of the somatotropin solution. The buffer can be Tris HCl, phosphate, or some other neutral, host-compatible pH buffer. Generally, the buffer is present in a concentration ranging from about 0.2 M to about 0.5 M. If Tris HCl is utilized as the buffer, the Tris HCl generally has a concentration of at least 0.2 M, and is preferably from about 0.2 M to about 0.3 M.

If the pH of the somatotropin composition prior to lyophilization was at least about 7.8, either a buffer as described above or a non-buffering agent can be added to the diluent, if desired, to adjust or maintain the isotonicity of the resultant somatotropin solution. The non-buffering agent also is host-compatible. Such

ag nts include sucrose, trehalose, or NaCl. Th non-buffering agent, if present, generally is provided at a concentration from about 0.05 M to about 0.5 M. When sucrose or trehalose is utilized as the non-buffering agent, preferred concentrations are within the range of from about 0.1 to about 0.3 M. When NaCl is utilized as the non-buffering agent a concentration of from 0.05 M to 0.15 M generally is preferred.

The somatotropin compositions of this invention can be made using kits containing a vial of the lyophilized somatotropin composition as discussed above and a vial containing the diluent discussed above. The diluent can be added to the lyophilized somatotropin composition and the resultant product shaken to dissolve the somatotropin.

The somatotropin solutions of this invention can be used for further processing or can be administered directly to animals. The solution generally can be stored for at least about 5 days at ambient temperatures, typically about 25°C, without becoming cloudy. The solutions also can be stored at slightly higher temperatures, although the solutions may maintain their clarity a shorter period of time. These solutions also can be subjected to mechanical agitation, such as shaking or mixing, without precipitation of the somatotropin from the solution. The somatotropin in these solutions maintains its biological activity and can be administered to animals in accordance with conventional techniques to promote growth.

The invention is further illustrated by the following examples, which are not intended to be limiting.

Examples

In the following examples, solution clarity was determined by visual inspection and/or by measuring the apparent absorbance at 360 nm (O.D. 360); a wavelength where somatotropins have no intrinsic absorbance. The somatotropin solution was placed in the sample cuvette and the O.D. 360 determined using a Shimadzu UVU160 spectrophotometer. A diluent solution which did not contain somatotropin was used in the reference cuvette. Quartz cuvettes were used in all studies. In the examples described, "clear" solutions are those which have an O.D. 360 of less than 0.10.

Example 1

Thirty-three grams of Zn-associated porcine somatotropin (pST), made in accordance with the procedures disclosed in published European Patent Application No. 83300803.9, and 90 grams of arginine HCl were dissolved in one liter of sterile water. The pH was adjusted to 7.8 by the addition of aqueous HCl or NaOH and insolubles were removed by filtration through a polyvinylidene difluoride membrane. The clarified solution then was lyophilized. The resulting formulation (termed the "lyophilized porcine somatotropin (pST) composition") contained approximately 30 g pST per 90 g arginine HCl, due to the loss of about 10% of the original 33 g of pST as insoluble during the filtration step.

Example 2

Solid arginine HCl (1.92 g) was added to a non-Zn complexed pST solution containing 0.639 g pST in 21.6 ml of a pH 9.8, 0.46 mM sodium carbonate buffer. The

arginine was dissolved by stirring and th pH of the solution was adjusted to 7.8 by the dropwise addition of 1 M NaOH. The resulting solution contained approximately 30 mg/ml pST and 90 mg/l arginine. Aliquots (7.2 ml) of this solution were pipetted into three 50-ml vials, frozen at -80°C, and lyophilized.

This lyophilized pST composition readily dissolved in 200 mM Tris HCl, 2 mM EDTA, 0.15% Brij 35 (pH 7.8) at 20 mg/ml (dissolution time < 3 minutes). The resulting solution was clear by visual inspection.

Example 3

The following solutions were prepared:

1. 0.2 M Tris HCl, 2 mM EDTA, 0.15% Brij 35, pH 7.8
2. " " " " " " " , pH 7.4
3. 2 mM EDTA, 0.15% Brij 35, pH 7.8

Zinc-associated pST (Zn-pST), non-metal complexed pST (non-Zn pST) and the lyophilized pST composition (made as described in Example 1) were dissolved by shaking for 2 minutes in each of the above solutions 5 mg/ml pST (or 20 mg/ml of the lyophilized pST composition). After the 2 minute shaking, solution clarity was determined by visual observation and by measuring turbidity at 360nm. As shown in Table 1, only the lyophilized pST composition gave a clear solution after dissolution under these conditions. The results are shown in Table 1.

Table 1

PST SOLUTION		OD360
Zn-pST	#1	0.522
"	#2	0.504
"	#3	0.381
non-Zn pST	#1	0.242
"	#2	0.347
"	#3	0.186
lyophilized pST composition	#1	0.019
"	#2	0.019
"	#3	0.019

Example 4

The following diluent solutions were made up:

1. 0.2M Tris HCl 2 mM EDTA pH 7.8 containing 0.15% Brij 35.
2. " " " " 0.125% " " .
3. " " " " 0.10% " " .
4. " " " " 0.08% " " .
5. " " " " 0.06% " " .
6. " " " " 0.04% " " .
7. " " " " 0.00% " " .

Forty mg aliquots of lyophilized pST composition (made as described in Example 1) were weighed out into seven 13 X 100 test tubes and dissolved in 2 ml of each of the above diluents. The solutions then were subjected to mechanical agitation by vortexing on a Vortex-Genie mixer for 30 seconds at a setting of 4.5. Solution clarity was determined by visual inspection by O.D. 360. The results are shown on Table 2.

Table 2

Effect of various diluents on mechanical stability of reconstituted pST

Diluent Solution No.	Appearance	O.D. 360
1	Clear	0.040
2	Clear	0.047
3	Clear	0.072
4	Clear	0.092
5	sl. cloudy	0.257
6	sl. cloudy	0.484
7	v. cloudy	1.708

* sl. cloudy = slightly cloudy; v. cloudy = very cloudy

As shown in Table 2, the somatotropin solutions containing a Brij 35 concentration of at least 0.08% resulted in a clear solution after vortexing the lyophilized pST composition in the diluent under these conditions. The somatotropin solutions containing a Brij 35 concentration of less than 0.08% did not remain clear following mechanical agitation by vortex mixing.

Example 5

Forty mg aliquots of lyophilized pST composition were weighed into 2 dram glass vials and dissolved in the diluent containing 200 mM Tris HCl, 0.10% Brij 35 at the following EDTA concentrations and final pH:

1. 2.0 mM EDTA, pH 7.2
2. 0.25 mM " , pH 7.4
3. 0.50 mM " , "
4. 1.00 mM " , "
5. 1.50 mM " , "
6. 2.00 mM " , "
7. 0.25 mM " , pH 7.6
8. 0.50 mM " , "
9. 1.00 mM " , "
10. 1.50 mM " , "
11. 2.00 mM " , "
12. 0.25 mM " , pH 7.8
13. 0.50 mM " , "
14. 1.00 mM " , "
15. 1.50 mM " , "
16. 2.00 mM " , "

These samples were then placed in a 25°C constant temperature chamber and checked for visual clarity at various time points. Results are shown in Table 3.

Table 3

Effect of various diluents on solution stability of reconstituted pST

Solution #	Day 1	Day 5	Day 10
1	clear	clear	clear
2	sl. cloudy	sl. cloudy	v. cloudy
3	clear	cloudy	v. cloudy
4	clear	sl. cloudy	cloudy
5	clear	clear	clear
6	clear	clear	clear
7	clear	cloudy	v. cloudy
8	clear	sl. cloudy	v. cloudy
9	clear	clear	cloudy
10	clear	clear	clear
11	clear	clear	clear
12	clear	cloudy	v. cloudy
13	clear	clear	cloudy
14	clear	clear	sl. cloudy
15	clear	clear	clear
16	clear	clear	clear

sl. cloudy = slightly cloudy
v. cloudy = very cloudy

As demonstrated in Table 3, somatotropin solutions, with a pH from 7.2 to 7.6, containing an EDTA concentration of at least 1.5 mM resulted in a clear solution for at least 10 days after dissolution of the lyophilized pST composition in the diluent under these conditions. Further, somatotropin solutions with a pH of 7.6 or greater, containing an EDTA concentration of at least 1.0 mM, resulted in a clear solution for at least 5 days after dissolution of the lyophilized pST in the diluent under these conditions.

Example 6

Forty mg aliquots of lyophilized pST composition were weighed into 16 two-dram glass vials. Two ml of the following diluents were added:

1. 300 mM Tris HCl, 0.15% Brij 35, 1.5 mM EDTA, pH 7.4
2. 200 mM Tris HCl, " " " " "
3. 100 mM Tris HCl, " " " " "
4. 50 mM Tris HCl, " " " " "
5. 25 mM Tris HCl, " " " " "
6. 200 mM Tris HCl, " " 2.0 mM EDTA, pH 7.2
7. " " " , " " 1.5 mM EDTA, "
8. " " " , " " 1.0 mM EDTA, "
9. 10% sucrose, 0.15% Brij 35, 2 mM EDTA
10. 5% sucrose, " " , " "
11. 1% sucrose, " " , " "
12. 0.2 M NaCl, 0.15% Brij 35, 2 mM EDTA
13. 0.1 M NaCl, " " , " "
14. 0.0 M NaCl, " " , " "

The final pH of the samples after reconstitution with diluents 9-14 was ~7.8-7.9 (or approximately equal to the final adjusted pH of the pST/Arg solution just prior to lyophilization). These samples were incubated in a 25°C constant temperature chamber for six days after which turbidity (O.D. 360 nm) readings were taken. Results are shown in Table 4.

Table 4

Effect of various diluents on solution stability of reconstituted pST

Solution #	Day 6 (O.D. 360)
1	0.021
2	0.064
3	0.264
4	0.537
5	0.658
6	0.064
7	0.084
8	0.979
9	0.025
10	0.025
11	0.025
12	0.025
13	< 0.03
14	< 0.03

As demonstrated by solutions 1-5 in Table 4, a Tris HCl concentration of at least 200 mM must be employed in a diluent containing 0.15% Brij 35 and 1.5 mM EDTA at a pH of 7.4 in order for the somatotropin solution to remain clear for six days. Solutions 6-8 in Table 4 illustrated that an EDTA concentration of at least 1.5 mM was necessary, in addition to a Brij 35 concentration of 0.15% and a Tris HCl concentration of 200 mM at a pH of 7.2, in order for the somatotropin solution to remain clear for six days. As demonstrated by solutions 9-14, the somatotropin solution remain d clear after six days when the diluent comprised 0.15% Brij 35, 2 mM EDTA and either 1%, 5%, or 10% sucrose or 0.2 M, 0.1 M or 0.0 M NaCl and the final pH of the samples were approximately 7.8-7.9.

We claim

1. A somatotropin solution which remains clear for extended periods and when subjected to mechanical agitation comprising a lyophilized somatotropin composition comprising about 1 part somatotropin per 2 to 8 parts arginine HCl on a weight basis, the pH of which was about 7.2 to about 8.5 prior to lyophilization, which is dissolved in a diluent comprising EDTA, a nonionic surfactant, and, if the pH of the somatotropin composition prior to lyophilization was between 7.2 and 7.8, a buffer.

2. The somatotropin solution of claim 1, wherein the pH of the somatotropin composition prior to lyophilization was at least 7.8 and the diluent further comprises a host compatible non-buffering agent.

3. The somatotropin solution of claim 1, wherein the pH of the somatotropin composition prior to lyophilization was at least 7.8 and the diluent further comprises a host-compatible buffer.

4. The somatotropin solution of claim 1, 2, or 3 wherein the somatotropin is natural or recombinant bovine, porcine, human, avian, ovine, or equine somatotropin or a bioactive fragment or analog thereof.

5. The somatotropin solution of claim 4 wherein the somatotropin is pST.

6. The somatotropin of claim 5 wherein the pST is delta 7 pST.

7. The somatotropin of claim 4 wherein the somatotropin is a metal associated somatotropin.

8. The somatotropin solution of claim 1, 2, or 3 wherein the nonionic surfactant is polyoxyethylene-23 lauryl ether (Brij 35), Tween 80, or polyoxyethylene-20 cetyl ether (Brij 58) present in a concentration of from 0.08% to 2.0%.

9. The somatotropin solution of claim 8 wherein th nonionic surfactant is Brij 35 having a concentration of at least 0.08%.

10. The somatotropin solution of claim 9 wherein the nonionic surfactant is Brij 35 having a concentration of from about 0.1% to about 0.2%.

11. The somatotropin solution of claim 8 wherein the nonionic surfactant is Tween 80 or Brij 58 having a concentration of from about 0.08% to about 1.0%.

12. The somatotropin solution of claim 1 or 2 wherein the buffer is a neutral pH buffer present in a concentration of from 0.2 to 0.5 M.

13. The somatotropin solution of claim 12, wherein the buffer is Tris HCl or phosphate buffer.

14. The somatotropin solution of claim 13 wherein the buffer is Tris HCl having a concentration of from about 0.2 M to about 0.3 M.

15. The somatotropin solution of claim 3 wherein the concentration of the non-buffering component is from about 0.1 M to about 0.5 M.

16. The somatotropin solution of claim 15 wherein the non-buffering component is sucrose, trehalose, or NaCl.

17. The somatotropin solution of claim 1, 2, or 3 wherein the EDTA is present at a concentration of at least 1.5 mM if the pH of the somatotropin solution is less than 7.6, and is present in the diluent at a concentration of at least 1.0 mM if the pH of the somatotropin solution is at least 7.6.

18. A kit for making a somatotropin solution which remains clear when subjected to mechanical agitation and which does not become cloudy for extended periods, comprising

a vial containing a lyophilized somatotropin composition which comprises about 1 part somatotropin per 2 to 8 parts arginine HCl on a weight basis, wherein the pH of said somatotropin composition prior to lyophilization was within the range of about 7.2 to about 8.5; and

a vial containing a diluent comprising EDTA, a nonionic surfactant, and, if the pH of the somatotropin composition prior to lyophilization was between 7.2 and 7.8, a buffer.

19. A kit in accordance with claim 18, wherein the pH of the somatotropin composition prior to lyophilization was at least 7.8 and the diluent further comprises a biocompatible non-buffering agent.

20. A kit in accordance with claim 18, wherein the pH of the somatotropin composition prior to lyophilization was at least 7.8 and the diluent further comprises a host-compatible buffer.

21. A kit in accordance with claim 18, 19, or 20 wherein from about 0.5 mg to about 40 mg lyophilized somatotropin composition is provided per ml of diluent.

22. A kit in accordance with claim 18, 19, or 20 wherein the somatotropin is natural or recombinant bovine, porcine, human, avian, ovine, or equine somatotropin or a bioactive fragment or analog thereof.

23. A kit in accordance with claim 18, 19, or 20 wherein the surfactant is polyoxyethylene-23 lauryl ether (Brij 35), Tween 80, or polyoxyethylene-20 cetyl ether present in a concentration from 0.08% to 2.0%.

24. A kit in accordance with claim 18 or 20 wherein the buffer is Tris HCl, phosphate, and is present in a concentration of from 0.2 M to 0.5 M.

25. A kit in accordance with claim 19 wherein the non-buffering component is sucrose, trehalose or

NaCl having a concentration of from about 0.1 M to about 0.5 M.

26. A process for making a somatotropin solution which remains clear when subjected to mechanical agitation and which remains clear for extended period which comprises the steps of:

(1) preparing a lyophilized somatotropin composition by dissolving arginine HCl with somatotropin in an amount such that there is 1 part somatotropin per 2 to 8 parts arginine HCl, adjusting the pH to within the range of about 7.2 to about 8.5, removing any undissolved material by filtration or centrifugation, and lyophilizing the resultant solution; and

(2) dissolving the resulting lyophilized somatotropin composition in a diluent comprising EDTA and a nonionic surfactant, wherein, if the pH of the somatotropin composition prior to lyophilization was between about 7.2 and about 7.8, the diluent further comprises a buffer.

27. A process in accordance with claim 26, wherein the pH of the somatotropin composition prior to lyophilization was at least 7.8 and the diluent further comprises a biocompatible non-buffering agent having a concentration within the range of about 0.1 M to about 0.5 M.

28. A process in accordance with claim 26, wherein pH of the somatotropin composition prior to lyophilization was at least 7.8 and the diluent further comprises a neutral, host-compatible buffer present in a concentration within the range of about 0.2 M to about 0.5 M.

29. A process in accordance with claim 26 or 28, wherein the buff r is Tris HCl or phosphate buffer.

30. A process in accordanc with claim 27, wherein the non-buff ring component is sucrose, trehalose or NaCl.

31. A process in accordance with claim 26, 27, or 28 wherein the somatotropin is natural or recombinant bovine, porcine, human, avian, ovine, or equine somatotropin or a bioactive fragment or analog thereof.

32. A process in accordance with claim 26, 27, or 28 wherein the nonionic surfactant is polyoxyethylene-23 lauryl ether (Brij 35), Tween 80, or polyoxyethylene-20 cetyl ether present in a concentration from 0.08% to 2.0%.

33. A method for promoting growth which comprises administering a growth-promoting amount of a somatotropin solution which comprises a lyophilized somatotropin composition comprising about 1 part somatotropin per 2 to 8 parts arginine HCl on a weight basis, wherein the pH of the somatotropin composition prior to lyophilization was about 7.2 to about 8.5, dissolved in a diluent comprising EDTA, a nonionic surfactant, and, if the pH of the somatotropin composition prior to lyophilization was less than about 7.8, a buffer.

34. A method for promoting growth which comprises administering a growth-promoting amount of a somatotropin solution which comprises a lyophilized somatotropin composition comprising about 1 part somatotropin per 2 to 8 parts arginine HCl on a weight basis, wherein the pH of the somatotropin composition prior to lyophilization was about 7.2 to about 8.5, dissolved in a diluent comprising EDTA, a nonionic surfactant, and, if the pH of the somatotropin prior to

lyophilization was at least 7.8, optionally further comprising a buffer or non-buffering agent.

International Application No PCT/US 93/03182

I. CLASSIFICATION F SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K37/02; A61K9/14; A61K47/18

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
Y	WO,A,9 014 100 (PITMAN-MOORE) 29 November 1990 see claims 1,4,6-10 see page 9, line 12 - line 31 ---	1-34
Y	WO,A,9 002 560 (PITMAN-MOORE) 22 March 1990 see claims 1,4,8-10,18-20 see page 6, line 28 - line 32 ---	1-34
Y	EP,A,0 303 746 (INTERNATIONAL MINERALS AND CHEMICAL CORPORATION) 22 February 1989 see claims 1-2,8,11-13 see page 4, line 35 - line 52 see example 1 --- -/--	1-34

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
02 AUGUST 1993	18. 08. 93
International Searching Authority	**Signature of Authorized Officer**
EUR PEAN PATENT FFICE	SCARPONI U.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	US,A,4 774 091 (Y.YAMAHIRA) 27 September 1988 see claims 1,5,9 see column 2, line 53 - line 68 see column 3, line 55 - line 61 see column 4, line 20 see example 5	1-34
Y	EP,A,0 131 864 (HOECHST) 23 January 1985 see claims 1,5,7,11-12 see page 4, line 15 - line 23 see page 5, line 27	1-34

INTERNATIONAL SEARCH REPORT | PCT/US 93/03182

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 33-34 are directed to a method of treatment of the human body by therapy (rule 39.1(iv) PCT), the search has been carried out and based on the alleged effects of the compositon.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] N protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9303182
SA 73272

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent ffice is in no way liable for these particulars which are merely given for the purpose of information. 02/08/93

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